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Review Article

Practical application of natriuretic peptides in paediatric cardiology

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Abstract It is still uncertain if cardiac natriuretic peptides are useful biomarkers in paediatric cardiology. In this review we identify four clinical scenarios in paediatric cardiology, where clinical decision-making can be difficult, and where we feel the paediatric cardiologists need additional diagnostic tools. Natriuretic peptide measurements could be that extra tool. We discuss and suggest N-terminal pro-B-type natriuretic peptide and B-type natriuretic peptide reference intervals for children without cardiovascular disease and cut-off points for the four specific paediatric heart conditions. We conclude that in premature neonates with persistent arterial ducts; in teenagers with tetralogy of Fallot and pulmonary regurgitation; and in children with heart transplants and potential allograft rejection cardiac peptides can provide the clinician with additional information, but in children with atrial septal defects the peptides are not helpful in guiding treatment or follow-up.

Keywords: B-type natriuretic peptide; N-terminal pro-B-type natriuretic peptide; congenital cardiac disease; heart transplantation; reference intervals

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In adult cardiology, natriuretic peptides are used as cardiac biomarkers while the use in paediatric cardiology is still questionable. Advances in medical imaging have greatly improved decision-making, but there still remain a number of relatively common problems where clinical examination and imaging are not always enough to plan treatment. Therefore, we have considered which paediatric heart conditions have a practical need for an additional tool and have chosen to investigate the role of cardiac natriuretic peptides in children with atrial septal defects, preterm babies with a patent arterial duct, children with tetralogy of Fallot with pulmonary regurgitation, and in diagnosis of rejection in children after heart transplantation. As a consequence some common paediatric heart defects are not discussed here, like ventricular septal defects where treatment can be guided by clinical examination and echocardiography, although cardiac peptides does correlate with the clinical presentation.[1] We have also reviewed the literature to establish valid reference intervals of B-type natriuretic peptide and N-terminal pro-B-type natriuretic peptide in healthy children and neonates.

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The cardiac natriuretic peptides

Almost 30 years ago, it was shown in rats that the heart is an endocrine organ with a hormonal link between the atria and kidneys.[2] A human equivalent identified as A-type or atrial natriuretic peptide and the structurally related B-type or brain natriuretic peptide are both cardiac hormones (Fig 1).[3,4] On chromosomal level, A-type and B-type natriuretic peptide genes are in close proximity on chromosome 1, while the C-type natriuretic peptide gene is located on chromosome 2.[5,6] The cardiac C-type

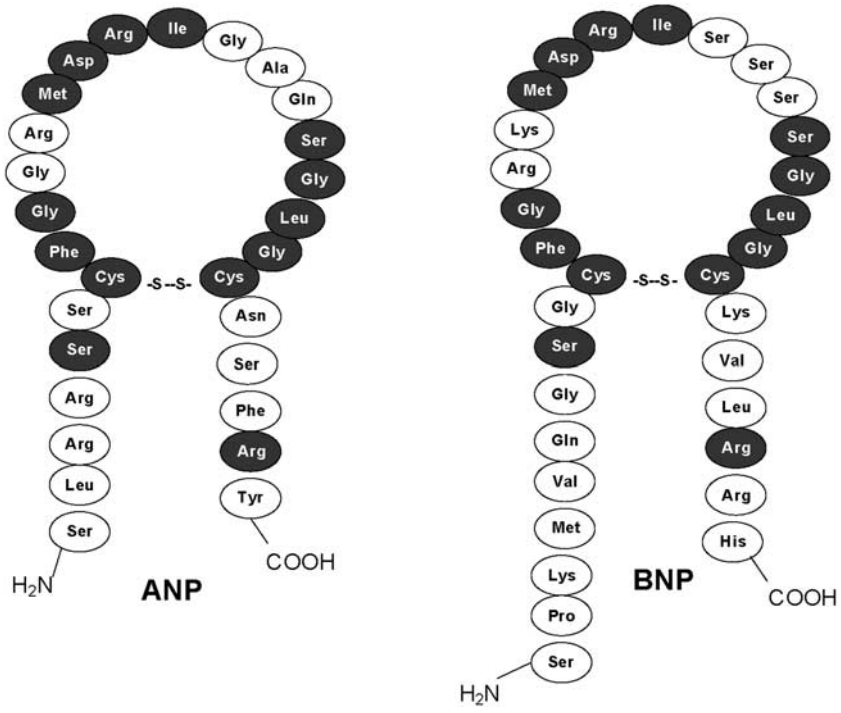

Figure 1.
A-type and B-type natriuretic peptides. The structural similarities between the bioactive peptides are primarily confined to the ring structure where 12, depictured in black, amino acid residues are identical. The ring structure is completed by a disulphide bridge from where N-terminal and C-terminal elements, the "tails", protrude from each of the cystyl residues.[3,86–88]

natriuretic peptide expression and secretion has for long been somewhat unclear and at best contradictory, but new research suggests it primarily to be a hormone involved in reproduction.[7] B-type and mainly A-type natriuretic peptides are expressed and stored in the atria of the normal adult heart. In the failing heart, A-type and B-type natriuretic peptide gene expression is dramatically regulated up in both atria and ventricles, and the total amount of B-type natriuretic peptide in the ventricles exceeds that of the atria. Human A-type natriuretic peptide expression appears to be developmentally regulated; foetal ventricles express larger amounts of mRNA compared with the adult myocardium and the expression decrease with gestational age. Comparable amounts of B-type natriuretic peptide mRNA are found in foetal and adult hearts.[8,9] However, in mice larger concentrations of B-type natriuretic peptides are found in foetal ventricles compared with adult ventricles, and peaks of A-type natriuretic peptide expression coincides with important stages of cardiac embryogenesis.[10] Mechanical stretch of the adult myocardium stimulates secretion of the peptides into the bloodstream and reactivates gene expression.[11–13] Consequently, systolic ventricular dysfunction increases ventricular release resulting in rise in B-type natriuretic peptide plasma concentrations.[14–17] Concentrations are also increased by hypoxia, tachycardia, and possibly cardiac fibrosis.[18–23] In addition, age, obesity, gender, and other hormonal and inflammatory mediators influence circulating concentrations.[24–28] B-type and A-type natriuretic peptides both cause hypotension and induce renal excretion of sodium and water.[29,30] B-type natriuretic peptide and the precursor-derived fragment N-terminal pro-B-type natriuretic peptide (Fig 2) are established biomarkers in adult cardiology.[31] Renal dysfunction can increase serum levels of cardiac peptides[32,33] and also pulmonary disease when it is associated with right ventricular dysfunction.[34] Immunoassays measure higher concentrations of N-terminal pro-B-type natriuretic peptide than B-type natriuretic peptide, but also one type of peptide can display significant concentration variations when using different kits.[35] As an example commercial B-type natriuretic peptide assays recognise not only the bioactive peptide, but also the shortened B-type natriuretic peptide 3-32, and commercial N-terminal pro-B-type natriuretic peptide assays also detect prohormone B-type natriuretic peptide 1-108 (Fig 2).[36–39] In addition, N-terminal pro-B-type natriuretic peptides can circulate in plasma as glycoproteins being difficult for the assays to recognise.[40] It is not known how age, gender, disease, genetics, or other factors influence the types of natriuretic peptides in plasma, and the respective physiological roles of the various types are unclear.

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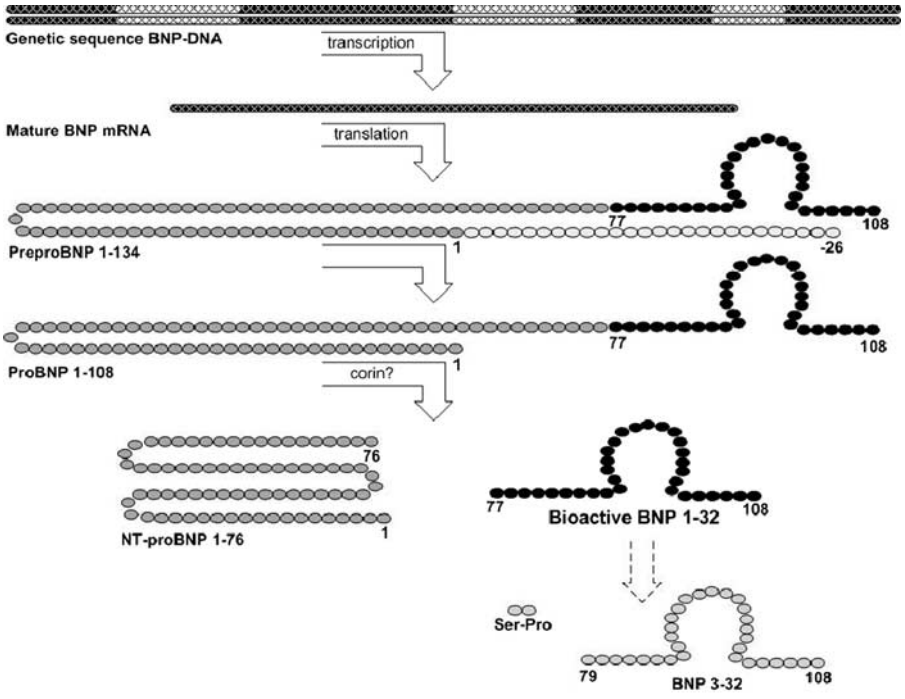

Figure 2.
From genetic code (DNA) to bioactive hormone, BNP 1-32. A schematic drawing of the peptide maturation process. The C-terminals are at the 108th amino acid and the N-terminal regions are at the other end of the peptides. In myocardial tissue the mRNA transcript, deriving from DNA exon regions, is translated into a preprohormone named prepro-B-type natriuretic peptide 1-134. Subsequently a small N-terminal signal sequence is removed during translation, leading to prohormone pro-B-type natriuretic peptide 1-108.[4,89] A protease, possibly corin, is facilitating the cleavage of pro-B-type natriuretic peptide 1-108 into the actual bioactive hormone B-type natriuretic peptide 1-32 and its split product N-terminal pro-B-type natriuretic peptide 1-76.[90] Pro-B-type, N-terminal pro-B-type, and B-type natriuretic peptides are all measurable in plasma. The bioactive peptide can be trimmed in the N-terminal region by aminopeptidases to B-type natriuretic peptide 3-32, but the biological significance of this trimming is still unknown.[91]

Natriuretic peptides in healthy infants

In order to use natriuretic peptides in paediatric cardiology valid reference intervals for healthy children of all ages needs to be defined. This section summarises the research on plasma concentrations of N-terminal pro-B-type and B-type natriuretic peptide in children without cardiovascular disease. N-terminal pro-B-type natriuretic peptide plasma concentrations are higher in newborn twins than singletons, with close coherence between twin siblings.[41] The concentrations of cardiac peptides are 10-fold higher in umbilical cord plasma compared with maternal blood, which implies that cardiac peptides in neonates are not derived from placental transfer.[41,42] The mean concentration of N-terminal pro-B-type natriuretic peptide peaks the first day after birth, is halved on the second day, and decreases further during the first week, but remains higher than mean concentration in umbilical cord plasma (Fig 3).[41–48] Within the first month *post partum* concentrations becomes lower than in cord plasma and continue to fall (except for an outlying mean at 13 years of age)[55] (Fig 4).[45–56] Upper range concentrations are used as reference intervals in this review (Figs 5 and 6). Plasma values convert from picomoles per litre to nanograms per litre by a factor 8.457 kilo Dalton. Only few studies include B-type natriuretic peptide in healthy children, yet plasma mean concentration follows a similar pattern to N-terminal pro-B-type the first days of life with a marked increase on the first day and on the second day of life it declines (Fig 7), although the upper range is peaking on the second day due to an outlier (Fig 8).[57–61] After a continuous decline within the first 2 weeks of life concentrations remains at a similar level until adulthood (Fig 9).[54,60–64] In adult subjects concentrations increase with age.[65,66] The concentration of 9.5 picomoles per litre, 33 nanograms per litre is used in this review as the maximum reference value for children older than 2 weeks (Fig 10).[60] For B-type natriuretic peptide, picomoles per litre converts to nanograms per litre by factor 3.464. The high peak in cardiac peptide plasma concentrations *post partum* appears to be a hormonal response to the change from the low resistance placental circuit, to an exclusively systemic circulation resulting in increased left ventricular afterload. The right ventricle also experience increased afterload following arterial duct closure when

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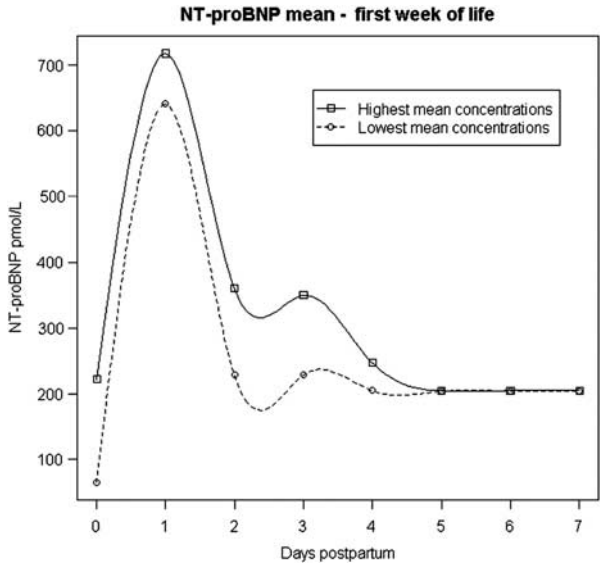

Figure 3.
The plots represent highest and lowest mean concentrations, picomoles per litre, in circulation found in studies of N-terminal pro-B-type natriuretic peptide the first 7 days of life.[41–48]

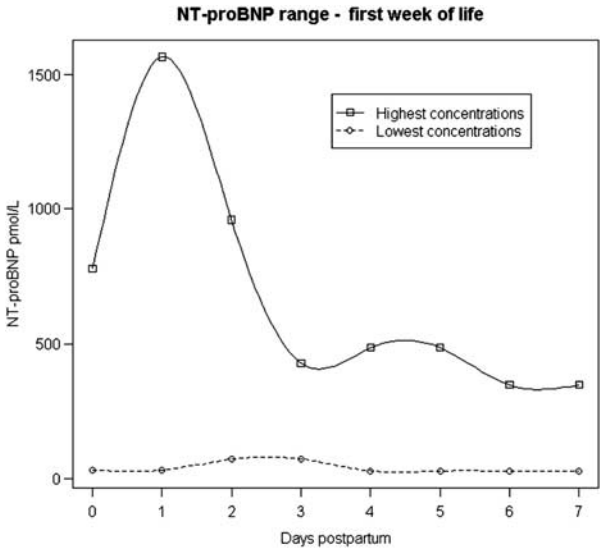

Figure 5.
The plots represent highest and lowest concentrations, picomoles per litre, in circulation found in studies of N-terminal pro-B-type natriuretic peptide the first 7 days of life.[41–48]

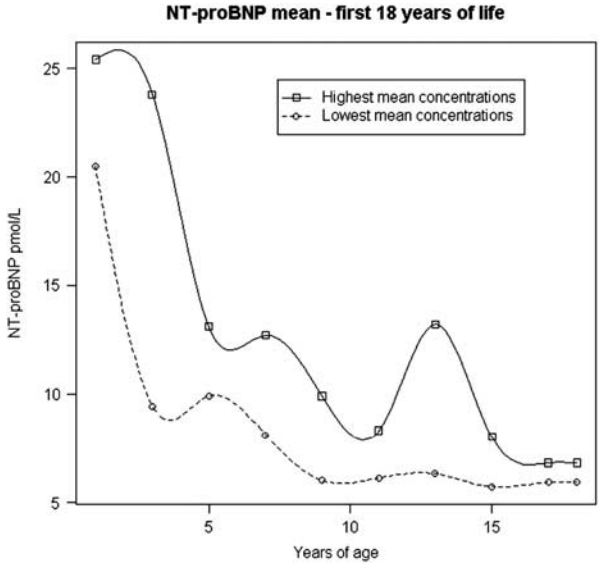

Figure 4.
The plots represent highest and lowest mean concentrations, picomoles per litre, in circulation found in studies of N-terminal pro-B-type natriuretic peptide from 10 days after delivery until 18 years of life.[46–56]

Figure 6.
The plots represent highest and lowest concentrations, picomoles per litre, in circulation found in studies of N-terminal pro-B-type natriuretic peptide from 10 days after delivery to 18 years of life.[48–56]

pulmonary vascular resistance is still high. Another theory is that cardiac peptide expression is unaffected by the cardiovascular changes after birth, but there is decreased clearance by the immature kidneys. However, neither hypothesis can explain the consistent decrease in plasma concentrations of N-terminal pro-B-type natriuretic peptide through childhood that is not seen for B-type natriuretic peptide (Figs 4, 6, 9, and 10).[54] N-terminal pro-B-type natriuretic peptide is able to conform to glycoprotein and this state is not fully detected by conventional assays;[40] so if age involves increasing amounts of glycosylation it could explain the decreasing concentrations. The half-life of the B-type is approximately 20 minutes, whereas half-life of the larger N-terminal pro-B-type natriuretic peptide is between 25 and 120 minutes;[67] this and

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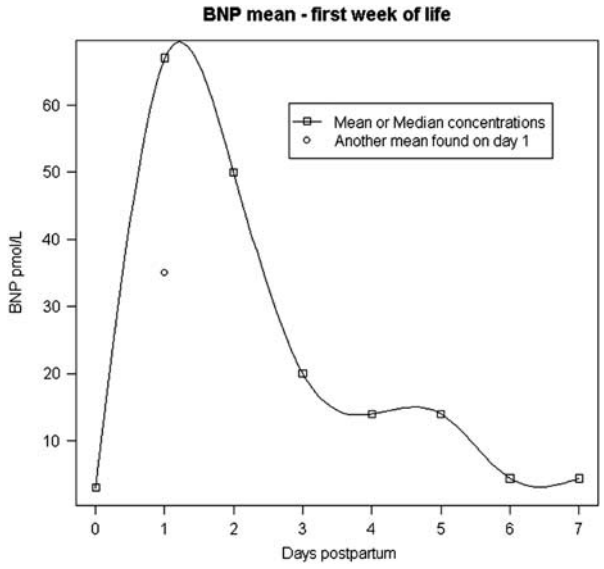

Figure 7.
The plots represent mean or median concentrations, picomoles per litre, in circulation found of B-type natriuretic peptide the first 7 days of life.[57–61] *Only one line was constructed due to very little available data on this subject.*

Figure 9.
Schematic drawing of mean B-type natriuretic peptide concentrations, picomoles per litre, in circulation from 1 to 17 years of life.[60–64]

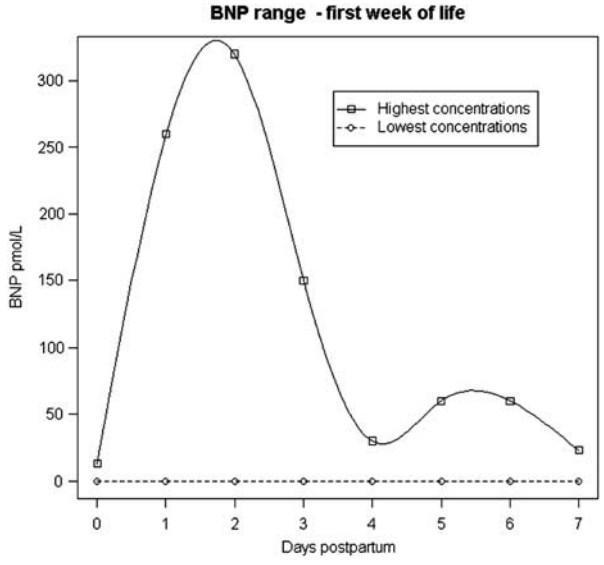

Figure 8.
The plots represent highest and lowest concentrations, picomoles per litre, in circulation found in studies of B-type natriuretic peptide the first 7 days of life.[57–61] *Notice the maximum plots peak on day 2 due to one extreme outlier in Koch and Singers study.*[60] *These lines are created from reading data plots in other studies due to little data available on this subject.*

Figure 10.
The plots represent highest and lowest concentrations, picomoles per litre, in circulation found in studies of B-type natriuretic peptide from 1 to 17 years of life.[60–64]

the glycosylation can explain the higher N-terminal pro-B-type concentrations compared with B-type natriuretic peptide. There is no gender difference in N-terminal pro-B-type concentrations,[46–55] but levels of B-type natriuretic peptide are higher in females than in males from 10 to 18 years of age (Fig 11).[60] The sex difference is suggested to be an inhibitory effect of testosterone in males rather than stimulatory by estradiol in females though this does not explain the higher concentration in adolescent girls compared with prepubertal girls.[60,68]

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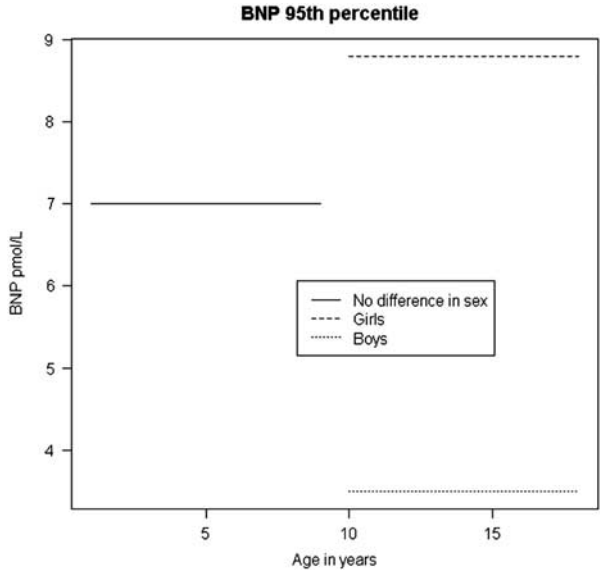

Figure 11.
The 95th percentile for B-type natriuretic peptide is depictured for all children from the first year to the 9th year of life, where there is no difference in gender. From 10 to 17 years the 95th percentile shows a significant difference between sex.[60] No difference in sex is found for N-terminal pro-B-type natriuretic peptide.

Four clinical problems in paediatric cardiology

We have identified four paediatric cardiac conditions where planning the correct treatment can be difficult even after thorough clinical examination and echocardiography. Atrial septal defects, persistent arterial ducts in preterm neonates, pulmonary regurgitation in tetralogy of Fallot, and cardiac allograft rejection are conditions where additional diagnostic tools are relevant.

The atrial septal defect

In foetal life, there is right to left shunting across the atrial septum through the foramen ovale. After birth, septum primum functions as a flap valve closing the atrial septum in response to increased left arterial pressure. If septum primum is insufficient the child is left with a secundum atrial septal defect, where blood will shunt from left to right. A large secundum defect is rarely a problem in young children, because right ventricular compliance is low in early life. As right ventricular compliance increases with age, left to right shunting increases and results in right ventricular volume overload. Most atrial septal defects will only be clinically significant in late childhood or adulthood, and sometimes only in old age. It is difficult to predict when or if a child will become symptomatic. A child who fails to thrive may be diagnosed with an atrial septal defect, but an echocardiogram will not always reveal if this is the cause of the problem and if an operation is needed. B-type natriuretic peptide correlates positively with the flow ratio between pulmonary and systemic blood flow (Qp/Qs) in patients with atrial septal defects.[61,69,70] Holmgren et al do not find this correlation, and the discrepancy is explained by "a narrow range of the left-to-right shunts, with the exception of one outlier".[62] B-type natriuretic peptide concentrations greater than 5.8 picomoles per litre (20 nanograms per litre) is suggested to identify patients with a significant shunt by Ozhan et al.[70] However, as seen in Figure 10, the highest concentration in healthy children older than 2 weeks without cardiac disease is 9.5 picomoles per litre, 33 nanograms per litre, hence the suggested cut-off point is within normal range and therefore not useful. Five studies of children with atrial septal defects measure B-type natriuretic peptide concentrations within and above normal range, 0–137 picomoles per litre; 0–473 nanograms per litre.[61,62,69–71] One would expect young children with atrial septal defects to have the lowest concentrations of cardiac peptides because low right ventricular compliance protects from left to right shunting through an atrial septal defect. However, this important physiological fact is not taken into account in existing studies, except for one, where younger patients have concentrations above normal, and older children's are within normal range,[71] but it only includes seven children, and the younger patients are probably diagnosed because they have very large septal defects, but unfortunately ratios of pulmonary and systemic flows are not measured. To decide if a moderate or large atrial septal defect is responsible for a child's failure to thrive remains a difficult call and evidence to date does not support use of cardiac peptides to guide the clinician. No studies have longitudinal measurements to potentially determine the need for operation if concentrations suddenly increase, and no study shows if closure of large atrial septal defects, benefit patients with high concentrations more than patients with low concentrations.

Persistent arterial duct in preterm neonates

A persistent arterial duct is a common problem in preterm neonates. Morbidity is high and can be associated with heart failure, intracranial haemorrhage, necrotising enterocolitis, and bronchopulmonary dysplasia when the duct is haemodynamically significant. It is not always obvious if premature neonates are in distress because of the persistent arterial duct or other complications associated with prematurity, therefore a serological marker to distinguish a minor from a

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haemodynamically significant persistent arterial duct is clinically relevant and lessens the need for numerous echocardiographies. Natriuretic peptide concentrations correlates with arterial duct size and shunt,[72–74] and are a potential screening tool to predict the need for medical[72,73,75,76] or surgical intervention.[75,76] The concentrations fall significantly when a haemodynamically significant duct is surgically closed or medically treated with success.[73,76] The clinical significance of persistent arterial ducts are closely related to changes in pulmonary vascular resistance, so when resistance is high even large ducts will not result in significant left to right shunting. Therefore, cardiac peptides ought to be within normal range, when pulmonary vascular resistance is high even if the arterial duct is large. However, both B-type and N-terminal pro-B-type natriuretic peptides measured on the second[72] or third day of life[72,75,76] have high sensitivity and specificity in predicting the need for duct closure with cut-off values of 160 picomoles per litre, 550 nanograms per litre, for B-type natriuretic peptide on the second day[72] and 320 picomoles per litre, 1110 nanograms per litre, on the third day of life.[74] For N-terminal pro-B-type natriuretic peptide concentrations above 1350 picomoles per litre, 11,395 nanograms per litre, on the third day *post partum* suggest the need for duct closure.[75] The above concentrations apply to preterm neonates of varying gestational ages, but no reference intervals are available. However, concentrations in preterm show the same changes as seen in term neonates with a peak the first day of life followed by decreasing concentrations.[75,77] Serial testing of cardiac peptides is uncomplicated when managing preterm infants, but it is the peptide values on the second and third day of life that appears to have the highest predictive value. In conclusion, B-type natriuretic peptide is a minimally invasive test, and values measured on the second and third day of life is helpful in determining whether a persistent arteria duct is or will become haemodynamically significant.

Pulmonary regurgitation and tetralogy of Fallot

In patients with tetralogy of Fallot and pulmonary stenosis the most common residual defect, after cardiac intervention or surgery, is pulmonary. Severe pulmonary regurgitation is a growing problem in paediatric cardiology, as more patients survive the initial treatment. Similar to atrial septal defects, pulmonary regurgitation is often not problematic in early childhood because the right ventricle has a relatively low compliance, but as compliance increases with age, regurgitation will increase and adversely affect right ventricular function. When interpreting cardiac peptide plasma concentrations in patients with repaired tetralogy of Fallot it is important to acknowledge the diverse clinical presentations. In uncorrected tetralogy of Fallot, concentrations are within normal range[78] so a hypertrophied right ventricle with systemic pressures does not appear to stimulate gene expression of the peptides. In surgically corrected tetralogy of Fallot, B-type natriuretic peptide concentrations can range from normal up to 254 picomoles per litre, 880 nanograms per litre,[69,71,79] and these values include patients with varying degrees of regurgitation. Right ventricular volume overload is common later in life causing exercise intolerance, atrial- and ventricular arrhythmias, the latter a potential cause of sudden death. Size and function of the right ventricle can be difficult to measure with routine echocardiography. Cardiac magnetic resonance imaging can measure right ventricular volumes and function accurately but is time consuming and costly, and there is no clear cut-off value for size and function when planning additional surgery. Consequently, a prognostic marker for right ventricular failure that could indicate when to insert a new pulmonary valve would be very helpful. Khositseth et al suggest that N-terminal pro-B-type natriuretic peptide concentrations above 13.6 picomoles per litre, 115 nanograms per litre, predicts right ventricular dilatation and dysfunction in children at average age 12.06 years plus or minus 2.54.[80] However, in healthy teenagers N-terminal pro-B-type natriuretic peptide concentrations range up to 46 picomoles per litre, 391 nanograms per litre, between 3 and 14 years of age and up to 27 picomoles per litre, 230 nanograms per litre, between 15 and 18 years of age (Fig 6), and therefore some patients with right ventricular dilatation and dysfunction have concentrations within normal range. Cheung et al[79] find positive correlation between B-type natriuretic peptide concentrations and the degree of regurgitation, average age 14.7 years, although Mir et al[71] find no correlation, average age 5.2 years. The most likely explanation for this discrepancy is difference in age between the subjects, because right ventricular dilatation normally is less pronounced in young children due to low right ventricular compliance even if pulmonary valve dysfunction is severe. Nevertheless, in adolescent patients with pulmonary regurgitation natriuretic peptide concentrations seem to correlate well with right ventricular dilatation.[79,80] Thus, cardiac peptides holds promise for predicting right ventricular overload in teenage Fallot patients, so when B-type natriuretic peptide concentrations are above 9.5 picomoles per litre, 33 nanograms per litre, or N-terminal pro-B-type natriuretic peptide concentrations are above 46 picomoles per litre, 391 nanograms per litre, in early teens or above 27 picomoles

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per litre, 230 nanograms per litre, in late teens (Figs 6 and 10) it warrants further investigation of right ventricular volume and function.

Heart transplantation and allograft rejection

It is difficult to determine clinically whether morbidity in paediatric heart transplant patients is caused by life threatening allograft rejection or by other childhood diseases. This is particularly an issue in local emergency departments or general paediatric wards where the clinician need to act quickly when an immunosuppressed heart transplant patient is unwell. Endomyocardial biopsy is gold standard for evaluating allograft rejection and all existing non-invasive techniques are considered less reliable. The natriuretic peptides are suggested as cardiac markers in paediatric transplant patients, where children with allograft rejection have a B-type natriuretic peptide mean concentration of 251 picomoles per litre, 870 nanograms per litre, before treatment, and 2.5 months after antirejection therapy it decreases to 42 picomoles per litre, 145 nanograms per litre.[81] Claudius et al find B-type natriuretic peptide concentrations range from 64 to 375 picomoles per litre, 221–1300 nanograms per litre, in nine plasma samples from paediatric heart transplant recipients with allograft pathology, but only 6–84 picomoles per litre, 20–290 nanograms per litre, in 50 recipients with no allograft pathology.[82] Most of the children with allograft pathology have rejection, but some have vascular diseases or left ventricular dysfunction, and all require special attention by transplant specialists. The study suggests concentrations below 29 picomoles per litre, 100 nanograms per litre, to exclude allograft pathology.[82] After 4–6 weeks post-heart transplantation children have concentrations up to 375 picomoles per litre, 1300 nanograms per litre, but 14 weeks after transplantation concentrations are less than 29 picomoles per litre, 100 nanograms per litre, in children with no rejection pathology.[83] Similarly, Rossano et al find the risk of allograft pathology is less than 1% when B-type natriuretic peptide concentrations are less than 29 picomoles per litre, 100 nanograms per litre, 1 year and above post-transplant.[84] In 53 paediatric transplant patients visiting the emergency room or urgent care unit B-type natriuretic peptide concentrations above 202 picomoles per litre, 700 nanograms per litre, suggests allograft rejection with a 100% sensitivity, 92% specificity and with a negative predictive value of 100%.[85] This suggested threshold includes all of the patients with acute allograft rejection in this particular study; however, other studies have measured concentrations below 202 picomoles per litre, 700 nanograms per litre, in patients with acute allograft pathology.[82,84] Even so, cardiac peptides are promising in diagnosing or excluding paediatric allograft rejection in the emergency room. In conclusion, paediatric heart transplantation recipients without rejection can have higher natriuretic peptide concentrations compared with normal healthy children. In the first weeks following heart transplantation, natriuretic peptide concentrations are very high where there is no difference between the rejection and non-rejection groups, but if a child is acutely ill more than 1 year after transplantation and plasma B-type natriuretic peptide is below 29 picomoles per litre, 100 nanograms per litre, then there is less than 1% chance of acute allograft rejection, but if the concentration is above 202 picomoles per litre, 700 nanograms per litre, then allograft rejection can be expected. B-type natriuretic peptide values between 29 and 202 picomoles per litre, 100 and 700 nanograms per litre, do not help the clinician in differentiating allograft pathology from other paediatric conditions.

Conclusion

There are several conditions in paediatric cardiology where clinical decision-making is difficult despite modern imaging technology and good clinical skills. We have listed four common clinical problems where additional tests are called for, and we have reviewed the role of cardiac natriuretic peptides as an additional tool to help the clinician. Reference intervals for natriuretic peptides are defined in this review based on published studies, but these are less well defined compared with reference intervals in adult patients. The presentation of congenital cardiac disease changes with age reflecting changes in the pulmonary vascular resistance and right ventricular compliance, a fact that has confounded many of the attempts to describe natriuretic peptide concentrations in children with cardiac disease. In preterm neonates with persistent arterial ducts, the natriuretic peptide concentrations on day 2 and 3 predict the need for duct closure. In children with heart transplants, concentrations help to guide treatment; and in teenagers with tetralogy of Fallot with pulmonary regurgitation, they imply the need for further investigations of right ventricular size and function. But in children with atrial septal defect, cardiac peptides have no clinical value when planning therapy.

Acknowledgement

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References

1. Suda K, Matsumura M, Matsumoto M. Clinical implication of plasma natriuretic peptides in children with ventricular septal defect. Pediatr Int 2003; 45: 249–254.
2. de Bold AJ, Borenstein HB, Veress AT, Sonnenberg H. A rapid and potent natriuretic response to intravenous injection of atrial myocardial extract in rats. Reprinted from Life Sci. 28: 89–94, 1981. J Am Soc Nephrol 2001; 12: 403–409.
3. Kangawa K, Matsuo H. Purification and complete amino acid sequence of alpha-human atrial natriuretic polypeptide (alpha-hANP). Biochem Biophys Res Commun 1984; 118: 131–139.
4. Sudoh T, Maekawa K, Kojima M, et al. Cloning and sequence analysis of cDNA encoding a precursor for human brain natriuretic peptide. Biochem Biophys Res Commun 1989; 159: 1427–1434.
5. Ogawa Y, Itoh H, Yoshitake Y, et al. Molecular cloning and chromosomal assignment of the mouse C-type natriuretic peptide (CNP) gene (Nppc): comparison with the human CNP gene (NPPC). Genomics 1994; 24: 383–387.
6. Tamura N, Ogawa Y, Yasoda A, et al. Two cardiac natriuretic peptide genes (atrial natriuretic peptide and brain natriuretic peptide) are organized in tandem in the mouse and human genomes. J Mol Cell Cardiol 1996; 28: 1811–1815.
7. Nielsen SJ, Gotze JP, Jensen HL, Rehfeld JF. ProCNP and CNP are expressed primarily in male genital organs. Regul Pept 2008; 146: 204–212.
8. Tsuchimochi H, Kurimoto F, Ieki K, et al. Atrial natriuretic peptide distribution in fetal and failed adult human hearts. Circulation 1988; 78: 920–927.
9. Takahashi T, Allen PD, Izumo S. Expression of A-, B-, and C-type natriuretic peptide genes in failing and developing human ventricles. Correlation with expression of the Ca(2+)-ATPase gene. Circ Res 1992; 71: 9–17.
10. Cameron VA, Aitken GD, Ellmers LJ, Kennedy MA, Espiner EA. The sites of gene expression of atrial, brain, and C-type natriuretic peptides in mouse fetal development: temporal changes in embryos and placenta. Endocrinology 1996; 137: 817–824.
11. Mantymaa P, Vuolteenaho O, Marttila M, Ruskoaho H. Atrial stretch induces rapid increase in brain natriuretic peptide but not in atrial natriuretic peptide gene expression in vitro. Endocrinology 1993; 133: 1470–1473.
12. Kinnunen P, Vuolteenaho O, Uusimaa P, Ruskoaho H. Passive mechanical stretch releases atrial natriuretic peptide from rat ventricular myocardium. Circ Res 1992; 70: 1244–1253.
13. Liang F, Wu J, Garami M, Gardner DG. Mechanical strain increases expression of the brain natriuretic peptide gene in rat cardiac myocytes. J Biol Chem 1997; 272: 28050–28056.
14. Hosoda K, Nakao K, Mukoyama M, et al. Expression of brain natriuretic peptide gene in human heart. Production in the ventricle. Hypertension 1991; 17: 1152–1155.
15. Nakao K, Mukoyama M, Hosoda K, et al. Biosynthesis, secretion, and receptor selectivity of human brain natriuretic peptide. Can J Physiol Pharmacol 1991; 69: 1500–1506.
16. Mukoyama M, Nakao K, Hosoda K, et al. Brain natriuretic peptide as a novel cardiac hormone in humans. Evidence for an exquisite dual natriuretic peptide system, atrial natriuretic peptide and brain natriuretic peptide. J Clin Invest 1991; 87: 1402–1412.
17. Tulevski II, Groenink M, van Der Wall EE, et al. Increased brain and atrial natriuretic peptides in patients with chronic right ventricular pressure overload: correlation between plasma neurohormones and right ventricular dysfunction. Heart 2001; 86: 27–30.
18. Weidemann A, Klanke B, Wagner M, et al. Hypoxia, via stabilization of the hypoxia-inducible factor HIF-1alpha, is a direct and sufficient stimulus for brain-type natriuretic peptide induction. Biochem J 2008; 409: 233–242.
19. Khan AR, Birbach M, Cohen MS, et al. Chronic hypoxemia increases ventricular brain natriuretic peptide precursors in neonatal swine. Ann Thorac Surg 2008; 85: 618–623.
20. Goetze JP, Gore A, Moller CH, et al. Acute myocardial hypoxia increases BNP gene expression. FASEB J 2004; 18: 1928–1930.
21. Rankin AJ, Courneya CA, Wilson N, Ledsome JR. Tachycardia releases atrial natriuretic peptide in the anesthetized rabbit. Life Sci 1986; 38: 1951–1957.
22. Tsuruda T, Boerrigter G, Huntley BK, et al. Brain natriuretic peptide is produced in cardiac fibroblasts and induces matrix metalloproteinases. Circ Res 2002; 91: 1127–1134.
23. Walther T, Klostermann K, Heringer-Walther S, et al. Fibrosis rather than blood pressure determines cardiac BNP expression in mice. Regul Pept 2003; 116: 95–100.
24. Mehra MR, Uber PA, Park MH, et al. Obesity and suppressed B-type natriuretic peptide levels in heart failure. J Am Coll Cardiol 2004; 43: 1590–1595.
25. Jensen KT, Carstens J, Ivarsen P, Pedersen EB. A new, fast and reliable radioimmunoassay of brain natriuretic peptide in human plasma. Reference values in healthy subjects and in patients with different diseases. Scand J Clin Lab Invest 1997; 57: 529–540.
26. Piuhola J, Szokodi I, Ruskoaho H. Endothelin-1 and angiotensin II contribute to BNP but not c-fos gene expression response to elevated load in isolated mice hearts. Biochim Biophys Acta 2007; 1772: 338–344.
27. Wiese S, Breyer T, Dragu A, et al. Gene expression of brain natriuretic peptide in isolated atrial and ventricular human myocardium: influence of angiotensin II and diastolic fiber length. Circulation 2000; 102: 3074–3079.
28. Emdin M, Passino C, Prontera C, et al. Cardiac natriuretic hormones, neuro-hormones, thyroid hormones and cytokines in normal subjects and patients with heart failure. Clin Chem Lab Med 2004; 42: 627–636.
29. Holmes SJ, Espiner EA, Richards AM, Yandle TG, Frampton C. Renal, endocrine, and hemodynamic effects of human brain natriuretic peptide in normal man. J Clin Endocrinol Metab 1993; 76: 91–96.
30. Misono KS, Grammer RT, Fukumi H, Inagami T. Rat atrial natriuretic factor: isolation, structure and biological activities of four major peptides. Biochem Biophys Res Commun 1984; 123: 444–451.
31. Clerico A, Iervasi G, Del Chicca MG, et al. Circulating levels of cardiac natriuretic peptides (ANP and BNP) measured by highly sensitive and specific immunoradiometric assays in normal subjects and in patients with different degrees of heart failure. J Endocrinol Invest 1998; 21: 170–179.
32. Buckley MG, Sagnella GA, Markandu ND, Singer DR, MacGregor GA. Immunoreactive N-terminal pro-atrial natriuretic peptide in human plasma: plasma levels and comparisons with alpha-human atrial natriuretic peptide in normal subjects, patients with essential hypertension, cardiac transplant and chronic renal failure. Clin Sci (Lond) 1989; 77: 573–579.
33. Buckley MG, Sethi D, Markandu ND, et al. Plasma concentrations and comparisons of brain natriuretic peptide and atrial natriuretic peptide in normal subjects, cardiac transplant recipients and patients with dialysis-independent or dialysis-dependent chronic renal failure. Clin Sci (Lond) 1992; 83: 437–444.
34. Nagaya N, Nishikimi T, Okano Y, et al. Plasma brain natriuretic peptide levels increase in proportion to the extent of right ventricular dysfunction in pulmonary hypertension. J Am Coll Cardiol 1998; 31: 202–208.
35. Goetze JP, Dahlstrom U, Rehfeld JF, Alehagen U. Impact of epitope specificity and precursor maturation in pro-B-type natriuretic peptide measurement. Clin Chem 2008; 54: 1780–1787.

36. Heublein DM, Huntley BK, Boerrigter G, et al. Immunoreactivity and guanosine 3',5'-cyclic monophosphate activating actions of various molecular forms of human B-type natriuretic peptide. Hypertension 2007; 49: 1114–1119.
37. Lam CS, Burnett JC Jr, Costello-Boerrigter L, Rodeheffer RJ, Redfield MM. Alternate circulating pro-B-type natriuretic peptide and B-type natriuretic peptide forms in the general population. J Am Coll Cardiol 2007; 49: 1193–1202.
38. Hawkridge AM, Heublein DM, Bergen III HR, et al. Quantitative mass spectral evidence for the absence of circulating brain natriuretic peptide (BNP-32) in severe human heart failure. Proc Natl Acad Sci U S A 2005; 102: 17442–17447.
39. Seferian KR, Tamm NN, Semenov AG, et al. The brain natriuretic peptide (BNP) precursor is the major immunoreactive form of BNP in patients with heart failure. Clin Chem 2007; 53: 866–873.
40. Seferian KR, Tamm NN, Semenov AG, et al. Immunodetection of glycosylated NT-proBNP circulating in human blood. Clin Chem 2008; 54: 866–873.
41. Hammerer-Lercher A, Puschendorf B, Sommer R, et al. Natriuretic peptides correlate between newborn twins but not between twins and their mothers. Clin Chim Acta 2007; 377: 279–280.
42. Hammerer-Lercher A, Mair J, Tews G, Puschendorf B, Sommer R. N-terminal pro-B-type natriuretic peptide concentrations are markedly higher in the umbilical cord blood of newborns than in their mothers. Clin Chem 2005; 51: 913–915.
43. Bakker J, Gies I, Slavenburg B, et al. Reference values for N-terminal pro-B-type natriuretic peptide in umbilical cord blood. Clin Chem 2004; 50: 2465.
44. Bar-Oz B, Lev-Sagie A, Arad I, Salpeter L, Nir A. N-terminal pro-B-type natriuretic peptide concentrations in mothers just before delivery, in cord blood, and in newborns. Clin Chem 2005; 51: 926–927.
45. Mir TS, Laux R, Hellwege HH, et al. Plasma concentrations of aminoterminal pro atrial natriuretic peptide and aminoterminal pro brain natriuretic peptide in healthy neonates: marked and rapid increase after birth. Pediatrics 2003; 112: 896–899.
46. Nir A, Bar-Oz B, Perles Z, et al. N-terminal pro-B-type natriuretic peptide: reference plasma levels from birth to adolescence. Elevated levels at birth and in infants and children with heart diseases. Acta Paediatr 2004; 93: 603–607.
47. Rauh M, Koch A. Plasma N-terminal pro-B-type natriuretic peptide concentrations in a control population of infants and children. Clin Chem 2003; 49: 1563–1564.
48. Schwachtgen L, Herrmann M, Georg T, et al. Reference values of NT-proBNP serum concentrations in the umbilical cord blood and in healthy neonates and children. Z Kardiol 2005; 94: 399–404.
49. Albers S, Mir TS, Haddad M, Laer S. N-Terminal pro-brain natriuretic peptide: normal ranges in the pediatric population including method comparison and interlaboratory variability. Clin Chem Lab Med 2006; 44: 80–85.
50. Cohen S, Springer C, Avital A, et al. Amino-terminal pro-brain-type natriuretic peptide: heart or lung disease in pediatric respiratory distress? Pediatrics 2005; 115: 1347–1350.
51. Eerola A, Jokinen E, Boldt T, Pihkala J. The influence of percutaneous closure of patent ductus arteriosus on left ventricular size and function: a prospective study using two- and three-dimensional echocardiography and measurements of serum natriuretic peptides. J Am Coll Cardiol 2006; 47: 1060–1066.
52. Eerola A, Pihkala JI, Boldt T, et al. Hemodynamic improvement is faster after percutaneous ASD closure than after surgery. Catheter Cardiovasc Interv 2007; 69: 432–441.
53. Geiger R, Hammerer-Lercher A, Url C, et al. NT-proBNP concentrations indicate cardiac disease in pediatric patients. Int J Cardiol 2007; 123: 63–65.
54. Koch AM, Rauh M, Zink S, Singer H. Decreasing ratio of plasma N-terminal pro-B-type natriuretic peptide and B-type natriuretic peptide according to age. Acta Paediatr 2006; 95: 805–809.
55. Mir TS, Flato M, Falkenberg J, et al. Plasma concentrations of N-terminal brain natriuretic peptide in healthy children, adolescents, and young adults: effect of age and gender. Pediatr Cardiol 2006; 27: 73–77.
56. Hammerer-Lercher A, Geiger R, Mair J, et al. Utility of N-terminal pro-B-type natriuretic peptide to differentiate cardiac diseases from noncardiac diseases in young pediatric patients. Clin Chem 2006; 52: 1415–1419.
57. Ko HK, Lee JH, Choi BM, et al. Utility of the rapid B-type natriuretic peptide assay for detection of cardiovascular problems in newborn infants with respiratory difficulties. Neonatology 2008; 94: 16–21.
58. Reynolds EW, Ellington JG, Vranicar M, Bada HS. Brain-type natriuretic peptide in the diagnosis and management of persistent pulmonary hypertension of the newborn. Pediatrics 2004; 114: 1297–1304.
59. Mannarino S, Ciardelli L, Garofoli F, et al. Correlation between cord blood, perinatal BNP values and echocardiographic parameters in healthy Italian newborns. Early Hum Dev 2009; 85: 13–17.
60. Koch A, Singer H. Normal values of B type natriuretic peptide in infants, children, and adolescents. Heart 2003; 89: 875–878.
61. Kunii Y, Kamada M, Ohtsuki S, et al. Plasma brain natriuretic peptide and the evaluation of volume overload in infants and children with congenital heart disease. Acta Med Okayama 2003; 57: 191–197.
62. Holmgren D, Westerlind A, Lundberg PA, Wahlander H. Increased plasma levels of natriuretic peptide type B and A in children with congenital heart defects with left compared with right ventricular volume overload or pressure overload. Clin Physiol Funct Imaging 2005; 25: 263–269.
63. Muta H, Ishii M, Maeno Y, Akagi T, Kato H. Quantitative evaluation of the changes in plasma concentrations of cardiac natriuretic peptide before and after transcatheter closure of atrial septal defect. Acta Paediatr 2002; 91: 649–652.
64. Takeuchi D, Saji T, Takatsuki S, Fujiwara M. Abnormal tissue doppler images are associated with elevated plasma brain natriuretic peptide and increased oxidative stress in acute Kawasaki disease. Circ J 2007; 71: 357–362.
65. Alehagen U, Goetze JP, Dahlstrom U. Reference intervals and decision limits for B-type natriuretic peptide (BNP) and its precursor (Nt-proBNP) in the elderly. Clin Chim Acta 2007; 382: 8–14.
66. Redfield MM, Rodeheffer RJ, Jacobsen SJ, et al. Plasma brain natriuretic peptide concentration: impact of age and gender. J Am Coll Cardiol 2002; 40: 976–982.
67. Kroll MH, Twomey PJ, Srisawasdi P. Using the single-compartment ratio model to calculate half-life, NT-proBNP as an example. Clin Chim Acta 2007; 380: 197–202.
68. Chang AY, Abdullah SM, Jain T, et al. Associations among androgens, estrogens, and natriuretic peptides in young women: observations from the Dallas Heart Study. J Am Coll Cardiol 2007; 49: 109–116.
69. Lin NC, Landt ML, Trinkaus KM, et al. Relation of age, severity of illness, and hemodynamics with brain natriuretic peptide levels in patients <20 years of age with heart disease. Am J Cardiol 2005; 96: 847–850.
70. Ozhan H, Albayrak S, Uzun H, et al. Correlation of plasma B-type natriuretic peptide with shunt severity in patients with atrial or ventricular septal defect. Pediatr Cardiol 2007; 28: 272–275.
71. Mir TS, Falkenberg J, Friedrich B, et al. Levels of brain natriuretic peptide in children with right ventricular overload due to congenital cardiac disease. Cardiol Young 2005; 15: 396–401.
72. Czernik C, Lemmer J, Metze B, et al. B-type natriuretic peptide to predict ductus intervention in infants <28 weeks. Pediatr Res 2008; 64: 286–290.

73. Choi BM, Lee KH, Eun BL, et al. Utility of rapid B-type natriuretic peptide assay for diagnosis of symptomatic patent ductus arteriosus in preterm infants. Pediatrics 2005; 115: e255–e261.
74. Flynn PA, da Graca RL, Auld PA, Nesin M, Kleinman CS. The use of a bedside assay for plasma B-type natriuretic peptide as a biomarker in the management of patent ductus arteriosus in premature neonates. J Pediatr 2005; 147: 38–42.
75. Farombi-Oghuvbu IO, Matthews T, Mayne PD, Guerin H, Corcoran D. N-terminal pro-B-type natriuretic peptide: a measure of significant patent ductus arteriosus. Arch Dis Child Fetal Neonatal Ed 2008; 93: F257–F260.
76. Puddy VF, Amirmansour C, Williams AF, Singer DR. Plasma brain natriuretic peptide as a predictor of haemodynamically significant patent ductus arteriosus in preterm infants. Clin Sci (Lond) 2002; 103: 75–77.
77. da Graca RL, Hassinger DC, Flynn PA, et al. Longitudinal changes of brain-type natriuretic peptide in preterm neonates. Pediatrics 2006; 117: 2183–2189.
78. Koch A, Zink S, Singer H. B-type natriuretic peptide in paediatric patients with congenital heart disease. Eur Heart J 2006; 27: 861–866.
79. Cheung EW, Lam WW, Chiu CS, et al. Plasma brain natriuretic peptide levels, right ventricular volume overload and exercise capacity in adolescents after surgical repair of tetralogy of Fallot. Int J Cardiol 2007; 121: 155–162.
80. Khositseth A, Manop J, Khowsathit P, et al. N-terminal pro-brain natriuretic peptide as a marker in follow-up patients with tetralogy of Fallot after total correction. Pediatr Cardiol 2007; 28: 333–338.
81. Lindblade CL, Chun DS, Darragh RK, et al. Value of plasma B-type natriuretic peptide as a marker for rejection in pediatric heart transplant recipients. Am J Cardiol 2005; 95: 909–911.
82. Claudius I, Lan YT, Chang RK, Wetzel GT, Alejos J. Usefulness of B-type natriuretic peptide as a noninvasive screening tool for cardiac allograft pathology in pediatric heart transplant recipients. Am J Cardiol 2003; 92: 1368–1370.
83. Lan YT, Chang RK, Alejos JC, Burch C, Wetzel GT. B-type natriuretic peptide in children after cardiac transplantation. J Heart Lung Transplant 2004; 23: 558–563.
84. Rossano JW, Denfield SW, Kim JJ, et al. B-type natriuretic peptide is a sensitive screening test for acute rejection in pediatric heart transplant patients. J Heart Lung Transplant 2008; 27: 649–654.
85. Geiger M, Harake D, Halnon N, Alejos JC, Levi DS. Screening for rejection in symptomatic pediatric heart transplant recipients: the sensitivity of BNP. Pediatr Transplant 2008; 12: 563–569.
86. Flynn TG, de Bold ML, de Bold AJ. The amino acid sequence of an atrial peptide with potent diuretic and natriuretic properties. Biochem Biophys Res Commun 1983; 117: 859–865.
87. Kangawa K, Fukuda A, Matsuo H. Structural identification of beta- and gamma-human atrial natriuretic polypeptides. Nature 1985; 313: 397–400.
88. Kambayashi Y, Nakao K, Mukoyama M, et al. Isolation and sequence determination of human brain natriuretic peptide in human atrium. FEBS Lett 1990; 259: 341–345.
89. Hino J, Tateyama H, Minamino N, Kangawa K, Matsuo H. Isolation and identification of human brain natriuretic peptides in cardiac atrium. Biochem Biophys Res Commun 1990; 167: 693–700.
90. Yan W, Wu F, Morser J, Wu Q. Corin, a transmembrane cardiac serine protease, acts as a pro-atrial natriuretic peptide-converting enzyme. Proc Natl Acad Sci U S A 2000; 97: 8525–8529.
91. Brandt I, Lambeir AM, Ketelslegers JM, et al. Dipeptidyl-peptidase IV converts intact B-type natriuretic peptide into its des-SerPro form. Clin Chem 2006; 52: 82–87.